Takashi MIKAWA*: A taxonomic study on Japanese sporangiferous Mucorales (1)**

三川 隆*: 日本産胞子嚢性ケカビ目菌類の分類学的研究 (1)**

The Mucorales is characterized by the following morphological features: 1) the mycelia are coenocytic, 2) asexual reproduction is by means of non-motile spores which are formed in rod-like or sac-like sporangia, 3) sexual reproduction is by means of zygospores which are formed by the fusion of equal- or unequal-sized gametangia. According to Benjamin (1966, 1967), this order is divided into the following two groups on the basis of the manner of the formation of asexual reproductive organ; the sporangiferous Mucorales and merosporangiferous Mucorales. Most members of the former generally grow in soil, dung, litter and others, being dependent on the soluble part of the substrates for their nutrition and thus they are said to be saprophytic sugar fungi. However, a few species are facultative parasitic. In contrast, the latter is mostly parasitic on the mucoralean fungi.

Historical account

The taxonomic study of the Mucorales dates back to Fischer (1892). His system has been employed by many subsequent authors with certain modifications (Lendner, 1908; Zycha, 1935; Naumov, 1939). Naumov's classification was constructed by the use of the combination of both asexual and sexual reproductive characters. It was followed by Hesseltine (1955) and Hesseltine & Ellis (1973) with slight modifications. Zycha's one (1935) was based in the main on the asexual reproductive characters and was followed by Zycha *et al.* (1969). At species level, the taxa of the merosporangiferous Mucorales have been considerably clarified by the works made by many mycologists, notably Benjamin (1958-1965). In contrast, the taxonomy of the sporangiferous Mucorales is in considerable disagreement. This confusion may be explained partly by the facts that some species show wide ranges of the morphological variations under cultural conditions, and therefore, there

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are seemingly strain differences within a species. In addition to these facts, it may lend some support to this confusion that the most species have been defined on the basis of delicate morphological characters.

In Japan, the beginning of the investigations of the Mucorales dates back to the work of fermentation organisms and air borne fungi in the twentieth century. In a serial survey of the fermentation organisms, fermentation substrates such as Chinese yeasts or fermented foods were perceived as principal focus of their habitats by many workers, including Saito (1904a, 1905, 1907), Hanzawa (1912, 1915), Nakazawa (1909) and Yamazaki (1917, 1918a, 1918b, 1919a, 1919b). During these serial studies, some groups of the Mucorales were added to Japanese fungal flora. To our knowledge of the fungal flora, contributions from the aspects of air borne fungi have been mainly presented by Saito (1902, 1904b), Nakazawa (1917) and Saito & Okazaki (1938). In pararell with such approaches, some investigations of the fungal flora from soil have been done since 1919 by Yamamoto (1926, 1929, 1930), Sasaki & Nakane (1946), Otani *et al.* (1950, 1951) and Lee (1972). Recently, animal dungs, together with soils have been perceived in the study on the Mucorales. Outstanding contributions to this field of mycology are those of Indoh (1962a, 1962b, 1965), who described more than 20 species of the merosporangiferous Mucorales. Other notable works, paying attention mainly to the fungi inhabiting in soil and dung, have been made by several authors, including Kominami *et al.* (1952, 1954) and Kobayasi & Tubaki (1954). As the data have been accumulated, flora of the mucoralean fungi were compiled by some authors. Among them, Saito (1902) is the first to publish such a paper, in which he described five Japanese species in four genera, and pointed out the lack of detailed research on Japanese Mucorales. Later, Ito (1936) published "Mycological Flora of Japan" giving the description of 59 species in 17 genera that covered all the known species at that time from Japan and its adjacent regions. Saito (1948) presented a comprehensive keys to the families, genera and species of the order. Subsequently, Hara (1959) and Saito (1961) summarized the fungal flora of Japan and its adjacent regions, including 77 and 95 species in 11 and 14 genera of the Mucorales, respectively. Looking back to the studies of Japanese Mucorales on soil and dung, intensive work was made by Indoh (1962) in the way of a taxonomic study of the merosporangiferous Mucorales, but little has been done in that of the sporangiferous Mucorales, despite their wide distribution in soil and dung.

For obtaining a better picture of composition of the sporangiferous Mucorales, particularly those in soil and dung in our country, I have made my effort since 1973, and, eventually I accept 94 species in 27 genera of the sporangiferous Mucorales in the Japanese flora. In the present study, special emphases are placed on the following three points: 1) treatment of soil and dung as sources, 2) examination of morphological characters of the taxa on chemically defined cultural medium, 3) re-examination of the data on all the described species in Japan. Systematic account concerning respective taxa of this group will be given in each separate paper.

Materials and Methods

Collection Samples collected were mainly soil and dung. Other additional materials such as litter, humus, fallen flower and mushroom were also collected. All the samples collected were put into clean polyethelene bags and carried back to the laboratory as soon as possible. Dung samples were usually put in sterilized paper-envelopes and air-dried. In total, 730 samples were collected from various localities, from subarctic zone to subtropic zone of Japanese Islands.

Isolation For the isolation of fungi from the samples, a direct inoculation method was mainly employed. Direct method and soil plate method were also used as supplementary methods. Those methods are as follows:

Direct inoculation method: A small amount of soil, dung, litter and humus was placed onto the agar plate containing weak maize meal agar.

Direct method: Dung, fallen flower and mushroom were placed directly in Petri-dishes with a little sterile water if nessesary.

Soil plate method (Warcup, 1950): A small amount of soil was placed in a Petri-dish, and then cooled 1.0% malt extract agar was poured on it. Petri-dish was rotated gently before sodification of the agar.

Isolation was carried out within 10 days after collection of the samples. Each plate was incubated at 20°C for 7-30 days and observed at 3-4 day intervals under the low-power (×60 or ×150) microscope. A single sporangium or few spores from a single sporangium was picked up by a sterile needle under low-power magnification and transferred to another agar plate.

Cultivation Synthetic *Mucor* agar, SMA (Hesseltine, 1954), has been widely used as a standard medium for the taxonomic descriptions of the most of the sporagiferous Mucorales. The merits of this medium are as follows: 1) it is chemically defined, 2) it gives excellent growth and sporulation of the most of the sporangiferous Mucorales, 3) it gives clear cultural characters such as colony color and appearance of colony. However, there are some inconveniences in this medium: 1) it gives compact colony and crowded aerial mycelia, and therefore, it is difficult to observe the branching pattern of sporangiophores and to determine the presence or absence of stolons and rhizoids, 2) as soon as sporangia touch the agar surface where the vegetative hyphae do not yet reach, spores germinate to give small pin-point colonies. Sporangiophores, sporangia and spores derived from this pin-point colonies are abnormal in their morphologies as compared with those of the original colony. This phenomenon complicates the specific identification of the most of the sporangiferous Mucorales. From those reasons, in the present study, Miura's agar, LCA[1] (Miura & Kudo, 1970) was chosen as a standard medium for the taxonomic description. The medium has the following merits: 1) it is chemically defined, 2) it gives excellent growth and sporulation of this groups of the Mucorales, 3) it gives no pin-point colonies, 4) it is easy on this medium to determine the presence or absence of stolons and rhizoids and to observe the pattern of branching of sporangiophores. However, on this medium, colony color is not well differential, because colony spreads loosely over the surface of agar. Therefore, in order to check the colony color, SMA was used as a supplementary medium. In addition to these media, PDA was used for *Phycomyces*, and dung extract agar was used for *Pilobolus*, since these members did not always grow on the LCA, even if they grow, they failed to sporulate.

All the culture plate were incubated at 20°C for 7-10 days in the dark.

Microscope preparation The microscopic method used in the present study followed that of Miura (1973).

Identification The isolates were identified in most cases in the light of the original descriptions. If the original description could not be seen, identification depended on the recent authoritative descriptions or monographs. When it is difficult to make a satisfactory determination from the literatures alone, identification was made by comparison with those authentic or typical strains obtained from IFO or CBS. The check of all the species hitherto known from Japan depended only on the descriptions and illustrations given by individial authors, because there were few living materials or no permanent microscopic slides deposited. When the living materials are available, they were re-examined under culture.

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[1] LCA: glucose 1 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, KCl 0.2 g, $NaNO_3$ 2 g, yeast extract 0.2 g, agar 13 g, distilled water 1000 ml. pH of the medium is adjusted to 6.5-7.0 with KOH.

Species description presented in this study was mainly based on the materials isolated by myself. All drawings were made with the aid of a camera lucida. Color name were after "Today's color/300". The measurement given in the description based on 20-100 observations.

Morphological account

Morphological characters used as taxonomic criteria in the present study were examined chiefly by the aid of laboratory culture. The cultural studies were carried out using a chemically defined media, viz, LCA or SMA. Since it is well known by mycologists that the groups of the sporangiferous Mucorales show extensive morphological variations and exhibit overlapping taxonomical characters, special efforts have been made to determine the range of morphological variability using many fresh isolates for each species. As a result, the following morphological charcters are taken up as taxonomic criteria in the present study: colonies, vegetative hyphae, galls, stolons, rhizoids, gemmae, trophocysts, sporangiophores, branchlets, head vesicles, subsporangial vesicles, sporangia, sporangial walls, columellae, apophyses, sporangiola, monosporous sporangiola, sporangiospores, zygospores and suspensors. When there is necessity for discussion, these morphological characters will be explained regarding the usefulness in subsequent taxonomic descriptions, respectively.

Key to the families

Families of the sporangiferous Mucorales keyed out below followed Hesseltine (1955) with a little modification.

1. Suspensors of zygospores being H-like 2
1. Suspensors of zygospores being tong-like 3
2. Sporangia, sporangiola or monosporous sporagiola present, borne on the

tips of sporangiophores 4

2. Sporangia and sporangiola absent. Only monosporous sporangiola present, borne on head vesicles of sporangiophores ....Cunninghamellaceae
3. Trophocysts present or absent. Only sporangia present. Sporangiola and monosporous sporangiola absent ....Pilobolaceae
3. Trophocysts always absent. Sporangia, sporangiola or monosporous sporangiola present 5
4. Only sporangia present, nondecidious. Sporangiola or monosporous sporangiola absent ....Mucoraceae
4. Sporangia present or absent, if present, nondecidious. Either sporangiola or monosporous sporangiola always present, decidious ....Thamnidiaceae
5. Sporangia columellate. Sporangial walls splitting into two hemispherical shell. Head vesicles present ....Choanephoraceae
5. Sporangia usually not columellate, rarely columellate. Sporangial walls not splitting into two hemispherical shell. Head vesicles absent .... Mortierellaceae

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我国におけるケカビ目菌類の分類学的研究は主に醱酵及び Air-borne fungi の研究分野から進められたため，醱酵の基質（Chinese yeast など）が菌類の生育の場として強く着目され，この菌群の主要な生育の場である土壤，糞などに着目した研究は極めて少ない。分節胞子囊形成群の分類については印東（1962）による研究があげられるが，胞子囊形成群については充分な研究がないという現状である。

筆者は 1973 年以来，生育基質として主に土壤や糞に着目し，日本各地に胞子囊形成群の採集を行ない多数の菌株を収集した。これらの菌株の培養結果と従来の記録などに基づいて日本産の胞子囊形成群の記載分類学的研究を実施した。本報告はその第一報を構成するもので，研究歴史，研究方法及び科の階級の検索表を記述した。

○西洋松露の我国への渡来（小林義雄）Yosio KOBAYASI: When the european truffel was introduced to Japan as the food material ?

西洋松露（Truffel）がいつ頃食品として我国に伝えられたか，それに就いての詮索である。チーズが既に元禄時代の江戸城中でケンペルによって試食用に供され，或は南蠻時代に伝えられたかも知れないが，これに比べると西洋松露の方はずっと後世のことであろう。南方熊楠がトルーフルの名でこれを紹介しているが(雑誌 太陽 1923)，これは外国の文献と外国に於ける経験によって記述したにすぎない。川村清一博士がかって上野の精養軒で供されたと述べているが，これは大正年代以降のことである。思うに明治開化以来，宮中で開かれた公式の宴会では，恐らく貴重な食品としてこれが供されて居たと思われ，当時の記録をしらべれば面白い資料が発見出来そうである。ところで近ごろ，例の鹿鳴館時代（1883-1887）にそこで催された宴会のメニューの写しを，つれづれなる儘にながめて居ると次の料理名が目についた。

　煮込牛繊肉　松露野菜

この松露であるが，宴会の開かれたのは明治20年11月10日のことであり，日本の松露の旬とは，いささかはずれている。また味の極めて淡白なこれが牛肉の煮込みとは合ひそうもない。そこでこのメニューの日本文に対応する仏文を見ると，Filet de Baeuf à la Perigord とある。これで読めた。ペリゲーはフランス西南部の西洋松露の産地であり，上等な松露の異名ともなっているからである。100年近くも昔のメニューをながめ舌なめずりしても致し方ないので，この項は是迄。　（国立科学博物館）